# Overwintering Survival, Phenology, Voltinism, and Reproduction Among Different Populations of the Leaf Beetle *Diorhabda elongata* (Coleoptera: Chrysomelidae)

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**ABSTRACT**   The classical biological control program for exotic saltcedars (various *Tamarix* species and hybrids) has involved the assessment of different populations of the leaf beetle *Diorhabda elongata* (Brullé) s.l. that are promising for release in areas of North America that are located south of 37° N latitude. We report here the overwintering survival, phenology, and voltinism of four *D. elongata* populations (Tunisia, Crete, Uzbekistan, and Turpan) in eastcentral Texas. In addition, we studied their developmental and reproductive biology, which also included the previously released population from Fukang, China. Overwintering survival of the adult beetles of the Crete and Tunisia populations was 90–99 and 75%, respectively. The Uzbekistan and Turpan beetles had <31% overwintering survival. All *D. elongata* populations began ovipositing in late March. The Turpan beetle may produce three summer generations and ceased oviposition by September. The Crete beetle produced four summer generations plus a partial fifth generation and ceased ovipositing by mid-October. Both the Tunisia and Uzbekistan beetles produced five summer generations plus an unsuccessful partial sixth generation; oviposition extended into late November. Larval development and survival were generally similar among *D. elongata* populations. The Turpan and Fukang beetles had a shorter preoviposition period and produced more but smaller egg masses than the other beetle populations. However, this did not alter a female's lifetime fecundity and generally did not affect the innate capacity for increase compared with other populations. The Crete beetle seems to be the most promising for release in central Texas and points further south.

**KEY WORDS**   weed biological control, *Diorhabda elongata*, saltcedar, *Tamarix*

Saltcedars (*Tamarix* spp., Tamaricales: Tamaricaceae) were introduced from Eurasia and Africa into the United States and Mexico in the 1800s (Baum 1967, Crins 1989). These deciduous shrubs or small trees were used as ornamentals, for windbreaks and shade, and to prevent the erosion of stream banks. However, after 1920, saltcedar began invading riparian areas, shorelines of lakes and reservoirs, and desert springs in the western United States and northern Mexico (Robinson 1965, DiTomaso 1998, DeLoach et al. 2000). A complex of four or five species and various hybrids of saltcedar (Gaskin and Schaal 2002, 2003) are contributing to this increasingly economic and environmental problem (DeLoach et al. 2000, Zavaleta 2000).

A classical biological control program is currently ongoing against saltcedar. The leaf beetle *Diorhabda elongata* (Brullé) s.l. (Coleoptera: Chrysomelidae)

has been released into the field at several locations in the western United States beginning in 2001, with complete defoliation of saltcedar occurring at many of these sites (DeLoach et al. 2004). The earliest field releases involved *D. elongata* collected near Chilik, Kazakhstan (43.59° N, 78.25° E; released in Utah) and Fukang, China (44.17° N, 87.98° E; released in other areas). Its host specificity and various other biological traits have been documented (DeLoach et al. 2003, Lewis et al. 2003a, b , Cossé et al. 2005, Dudley and Kazmer 2005, Herrera et al. 2005). The Fukang/Chilik populations established well at all five U.S. release sites north of 38° N latitude. However, no establishment occurred at release sites near or south of 37° N latitude where summer daylengths do not exceed 14.5 h, the critical photoperiod for the Fukang population (DeLoach et al. 2004, Bean et al. 2007). Adult beetles entered a reproductive diapause in early summer and presumably depleted their fat body reserves and starved before the following spring (Lewis et al. 2003b). This lack of establishment led to the collection and assessment of additional populations of *D. elongata* that displayed shorter critical photoperiods for diapause induction and therefore were more likely to

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establish at more southern latitudes in North America (DeLoach et al. 2004). These populations were collected across a wide geographical area of Eurasia and North Africa and below 43° N latitude. All the *D. elongata* populations that we tested (Sfax, Tunisia; Crete, Greece; Karshi, Uzbekistan; and Turpan, China) are similar to each other in their host specificity (Milbrath and DeLoach 2006a, b) and have been approved for release. However, they may vary in other biological traits that could affect the likelihood of their establishment and subsequent increase.

We report here our evaluation of the overwintering survival, phenology, and voltinism of these four populations of *D. elongata* under seminatural conditions. We also studied aspects of their development and reproductive biology, which included some comparisons with the Fukang population. This information will aid in determining the most promising population(s) of *D. elongata* to release in the southern areas of the saltcedar infestation in North America.

## Materials and Methods

**Insect Colonies.** The four populations of *D. elongata* included in this study were collected near Sfax, Tunisia (34.66° N, 10.67° E); Sfakaki, Crete, Greece (35.83° N, 24.6° E); Karshi (Qarshi), Uzbekistan (38.86° N, 65.72° E); and Turpan, Xinjiang Province, China (42.86° N, 89.22° E). For one of the experiments, we included the initially released population from Fukang, China. We hereafter refer to these as *D. elongata* from Tunisia, Crete, Uzbekistan, Turpan, and Fukang. Voucher specimens of *D. elongata* were deposited with the National Collection of Insects and Mites of the National Museum of Natural History, Smithsonian Institution, Washington, DC. (under lot numbers GSWRL-2004-02 and -2005-02). Beetle colonies were maintained either on potted saltcedar plants in the USDA–ARS Arthropod Containment Facility (quarantine) at Temple, TX, or on saltcedar trees growing in field cages on the fenced grounds of the Temple laboratory under a permit from the USDA Animal and Plant Health Inspection Service.

**Overwintering Survival.** Overwintering survival of the Crete, Tunisia, Uzbekistan, and Turpan populations was assessed on the grounds of the Temple Laboratory (31.10° N, 97.36° W) as follows: 2002–2003, Crete; 2003–2004, Tunisia, Uzbekistan, and Turpan; 2004–2005, Crete, Tunisia, and Uzbekistan. Beetles were reared on saltcedar plants under natural daylengths. Beginning in late October or November, when saltcedar was naturally senescing, groups of three, four, or five unsexed, diapausing adults were placed in separate 240-ml plastic containers with drainage holes and covered with a mesh top. Containers held 3 cm of sand with a 3-cm layer of dried grass on top in which the adults could bury themselves. The containers were partially buried in the ground in outdoor 3 by 3 by 3-m (length by width by height of peaked roof) field cages and loosely covered by a shade cloth, with 14–16 containers per beetle population. Beetles normally overwinter as adults in the litter beneath saltcedar (DeLoach et al. 2004). Beetles were provided fresh saltcedar foliage every 3–4 d during the first month in the containers until no feeding was observed. The beetles experienced temperature and daylength variations comparable to those observed in nature. The respective mean minimum and maximum temperatures and temperature ranges for the three winters (December to mid-March) were as follows: 2002–2003, 5 and 16°C (range, −6 to 28°C); 2003–2004, 7 and 17°C (range, −4 to 25°C); and 2004–2005, 7 and 17°C (range, −6 to 28°C). To determine percentage survival, 5–10 containers per beetle population were randomly selected every 2–4 wk from December to the beginning of March, and the number of live and dead adults was counted. Their location in the cup (on top of or in the grass) was noted, dead adults were removed, and all containers were placed back outdoors. Beetles from all containers were assessed for survival on the last sampling date in mid-March, which coincided with the emergence of overwintered adults in separate outdoor colony cages. The experimental design for each of the latter 2 yr of this study involved a one-way treatment structure (beetle population) in a completely randomized design. There were 5–16 replicates depending on the sampling date. For each year, analysis of covariance (ANCOVA; PROC MIXED, SAS Institute 2004) was used to estimate and compare the regression parameters of percentage beetle survival (arcsine square root transformed) against time (d) for each population. Beetle population was the independent variable and time served as the covariate. The regression model was as follows: percentage survival = a + b × time. The parameters a (intercept) and b (slope) and the final percentage survival were compared among populations within years using preplanned contrasts (SAS Institute 2004).

**Phenology and Voltinism.** We observed the seasonal duration of beetle activity and estimated voltinism for each population of *D. elongata* by using a saltcedar grove established on the grounds of the Temple Laboratory. Field cages (3 by 3 by 3-m [length by width by height of peaked roof]) were erected over groups of saltcedar plants, each group containing one to two different *Tamarix* species or hybrids. Because of this layout, we could not rear all beetle populations on the same type of saltcedar. The area immediately surrounding the field cages was treated periodically with hydramethylnon (Amdro; Ambrands, Atlanta, GA) to control fire ant (*Solenopsis invicta* Buren; Hymenoptera: Formicidae) infestations.

Preliminary observations of beetle phenology and voltinism were made on field cage colonies during 2003. A more detailed study was conducted in 2004. Naturally overwintered adults of Crete, Uzbekistan, Tunisia, and Turpan beetles were obtained on termination of the previous 2003–2004 overwintering study or from outdoor colony cages. Groups of adults (1 male:1 female) were placed in either 12 by 25-cm (4 adults) or 30 by 55-cm (10 or 20 adults) polyester organza sleeve bags. The bags were tied to the branches of saltcedar plants growing within the large

field cages (Crete: *T. canariensis* Willldenow/*T. gallica* L. and *T. parviflora* de Candolle, remaining beetle populations: *T. ramosissima* Ledebour). Bags were checked periodically and the beginning and end of oviposition as well as adult mortality were noted. On first oviposition, which we considered the start of a new summer generation, a random selection of egg masses (≈50 eggs total) were transferred to a new 30 by 55-cm bag and tied to a saltcedar branch for rearing to adulthood. All subsequent egg masses found in the adult bags were destroyed. Because only the initial cohort of each summer generation was reared, we estimated the final date that larval and pupal stages and subsequent adult emergence would occur for each summer generation. This was done by using the linear degree-day model developed for *D. elongata* by Herrera et al. (2005) and temperature records for the site. On emergence of new adults from the first cohort of eggs, 10 male and 10 female beetles were randomly selected and placed in a new 30 by 55-cm bag. We repeated the rearing of the first 50 eggs and observations of the first 20 adults for each subsequent summer generation that was produced until adult beetles entered reproductive diapause and/or larvae could not complete their development because of senescence of saltcedar and the onset of cold temperatures in the fall.

**Larval Development and Adult Reproduction.** Two tests were conducted to compare survival, development, and reproduction among the different populations of *D. elongata*. Crete and Fukang beetles were compared in a test from August to October 2002, and a July to September 2003 test compared *D. elongata* from Tunisia, Uzbekistan, and Turpan. The experimental design for each year was a one-way treatment structure (beetle population) in a completely randomized design. Tests were conducted in the quarantine laboratory under fluctuating temperatures, both daily and longer term (average, 28°C; overall range, 23–33°C) and a photoperiod of 16:8 h (L:D). All populations for a given year were reared simultaneously.

In 2002, 12 groups of 10 first instars (0–15 h old) for both the Crete and Fukang populations were placed in separate 30 by 55-cm bags that were securely tied to the branches of potted saltcedar plants (*T. ramosissima*). The number of days to reach the adult stage was noted for 36–38 individuals (replicates) per population, and percentage survival to the adult stage was calculated for each group of larvae (12 replicates per population). For the 2003 test, 50 randomly selected larvae (0–15 h old) from each of the three beetle populations were placed in separate 50-ml ventilated, clear plastic vials. Larvae were fed excised foliage of saltcedar plants (*T. ramosissima*), and larvae or pupae were checked daily for survival and development. Sand was placed in the bottom of each vial when mature third instars (final instar) were present to provide a pupation site. Each larva served as a replicate (up to 50 per beetle population) for the duration of the various life stages. Percentage survival to the adult stage was calculated by randomly assigning 10

larvae to each of five groups (replicates) for each beetle population.

Adult *D. elongata* that eclosed were sexed using the characters of the last visible abdominal sternite (Lewis et al. 2003b). For both years, 20 mating pairs of recently eclosed adults (<24 h old) per beetle population were placed in separate 50-ml ventilated, clear plastic vials and fed excised saltcedar foliage. Adults were checked daily for survival and the presence of eggs. Males that died were replaced by similar-age males if available. Egg masses for each female were removed and placed in individual wells of marked well plates. The plates were covered with Parafilm (Pechiney Plastic Packaging, Chicago, IL), in which pin holes were made to provide ventilation, and held until hatching. The total number of eggs and hatched eggs per mass were recorded. The earliest egg masses produced were checked twice daily for hatching, and observations of 50 randomly selected individuals were used to estimate the number of days until hatch. The preoviposition and oviposition period, total fecundity, and fertility were calculated for each female. Females that escaped or were not fertile were not included in analyses. Total longevity was calculated for males and females. We used the data on development, survival and fecundity to calculate population growth statistics, including net reproductive rate ($R_0$), mean generation time (T), innate capacity for increase ($r_m$), and population doubling time (DT), using methods described by Birch (1948). Comparisons of population growth statistics were made by randomly dividing females into four groups (replicates) of four to five females for each of the beetle populations. Data from the two different years were analyzed separately. Analysis of variance (ANOVA; PROC MIXED; SAS Institute 2004) was performed on number of days development or duration for the various life stages, percentage survival (neonate to adult), total number of eggs and egg masses produced per female, number of eggs per mass, percentage egg hatch, and the four population growth statistics with beetle population as the independent variable. For adult longevity only, beetle population, sex and the interaction served as independent variables. Percentage data were arcsine square root transformed. Means were separated using Fisher's protected least significant difference (LSD) test (SAS Institute 2004).

We studied whether there was variation in egg mass size and differences in oviposition site (bark versus foliage) among *D. elongata* populations. This was done using previously unpublished data collected during host-specificity tests in outdoor cages (Milbrath and DeLoach 2006a, b). The average number of eggs per mass was calculated for the Crete, Tunisia, Uzbekistan, and Turpan beetles from the paired-choice test (*Tamarix: Frankenia*) of Milbrath and DeLoach (2006a). Each cage (replicate) represented the oviposition of up to 10 females on saltcedar plants (*T. ramosissima × T. chinensis*) over a 5-d period (13–162 egg masses per replicate). We also determined the percentage of eggs oviposited on the bark of saltcedar test plants (*T. ramosissima × T. chinensis*) as opposed
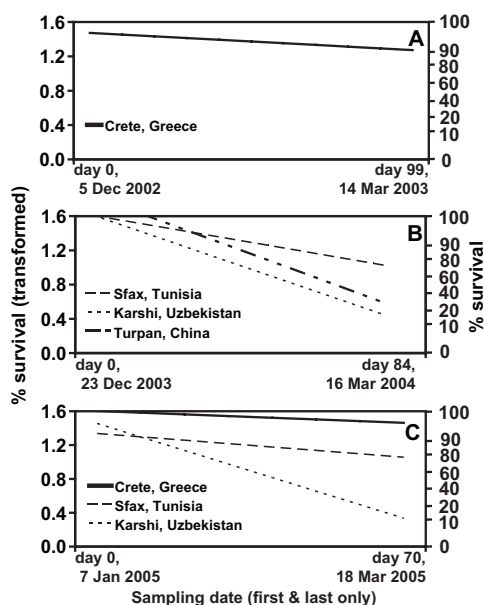
**Fig. 1.** Percentage overwintering survival (arcsine square root transformed, left axis; nontransformed, right axis) versus time (d) for *D. elongata* from Crete, Tunisia, Uzbekistan, and Turpan, in outdoor cages at Temple, TX. (A) 2002–2003. (B) 2003–2004. (C) 2004–2005. Only beginning and ending dates are shown for the regression lines. See Table 1 for analyses.

to the foliage in each cage from the 2003 paired-choice test (potential host:nonhost) of Milbrath and De-Loach (2006a) and the 2004 no-choice test of Milbrath and DeLoach (2006b). Eggs of *D. elongata* are usually laid directly on the foliage (Lewis et al. 2003b). As before, each cage (replicate) represented the oviposition of up to 10 females over a 3- or 5-d period (163–915 eggs per replicate). The experimental design for these data was a one-way treatment structure (beetle population) in a completely randomized design, with 2 to 10 replicates depending on the particular beetle population and data set. ANOVA (PROC MIXED; SAS Institute 2004) was performed on the number of eggs per mass and percentage of eggs ovi-

posited on saltcedar bark, with beetle population as the independent variable. Percentage data were arc-sine square root transformed. Means were separated using Fisher's protected LSD test (SAS Institute 2004).

## Results

**Overwintering Survival.** Overwintering survival of adult *D. elongata* in eastcentral Texas varied by beetle population but was fairly consistent for individual populations among years. Percentage survival decreased little over time for Crete *D. elongata* during the 2002–2003 winter, with 90% of the beetles surviving by mid-March 2003 (Fig. 1A). This date coincided with the spring growth of saltcedar leaves. During the winter of 2003–2004, survival over time was greater for the Tunisia beetle than the Turpan and Uzbekistan beetles and similar between the Turpan and Uzbekistan populations, as indicated by a comparison of the slopes (parameter b) of the regression lines (Fig. 1B; Table 1). By mid-March 2004, 75% of adult Tunisia beetles were still alive, which was much greater than the survival of the Turpan (31%) and Uzbekistan (16%) populations ($F = 19.33$; df = 1,137; $P < 0.001$ and $F = 34.14$; df = 1,137; $P < 0.001$, respectively). Percentage survival was similarly low for the Uzbekistan and Turpan beetles ($F = 3.21$; df = 1,137; $P = 0.075$). Most Uzbekistan and Turpan adults were present on top of the grass in the overwintering containers at the beginning of March 2004 when no green buds were present on saltcedar. This early spring activity was followed by mortality of most of the beetles.

Over the course of the 2004–2005 winter, the change in survival over time did not differ between the Crete and Tunisia beetles. However, both populations displayed higher survival rates than the Uzbekistan population (Fig. 1C; Table 1). The final percentage survival for Crete beetles was 99%, greater than that of the Tunisia beetle (75%; $F = 14.52$; df = 1,116; $P < 0.001$) and Uzbekistan beetle (10%; $F = 108.98$; df = 1,116; $P < 0.001$). Percentage survival of the Tunisia beetle also was much higher than the Uzbekistan beetle ($F = 44.76$; df = 1,116; $P < 0.001$).

**Table 1.** Parameter estimates for linear regressions describing percentage overwintering survival over time for four populations of *D. elongata*, Temple, TX

| Year of test<br>*D. elongata* population | Intercept (a) (±SE) | Slope (b) (±SE) | Residual mean-square error |
|---|---|---|---|
| 2002–2003 | | | 0.115 |
| Crete, Greece | 1.473 (0.094) | −0.002 (0.001) | |
| 2003–2004 | | | 0.122 |
| Sfax, Tunisia | 1.609a (0.121) | −0.007a (0.002) | |
| Karshi, Uzbekistan | 1.611a (0.121) | −0.014b (0.002) | |
| Turpan, China | 1.836a (0.097) | −0.015b (0.002) | |
| 2004–2005 | | | 0.113 |
| Crete, Greece | 1.603a (0.103) | −0.002a (0.002) | |
| Sfax, Tunisia | 1.337a (0.100) | −0.004a (0.002) | |
| Karshi, Uzbekistan | 1.455a (0.103) | −0.016b (0.002) | |

The linear regression was: % survival = a + b × time, where % survival was arcsine square root transformed and time is in days.
For each year and column, values denoted by the same letter are not significantly different (preplanned contrasts, $P > 0.05$).
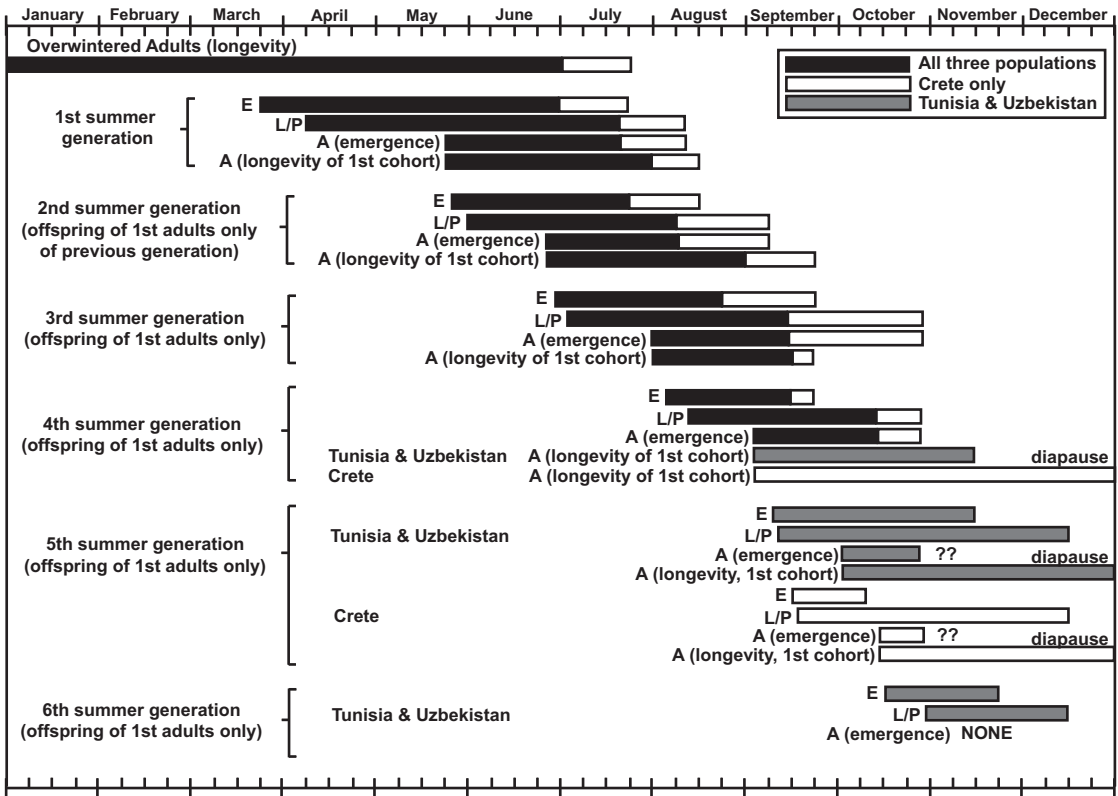
**Fig. 2.** Phenology and voltinism of *D. elongata* from Crete, Tunisia, and Uzbekistan reared in outdoor field cages at Temple, TX, 2004. E, eggs; L/P, larvae/pupae; A, adults. Black bars denote the observed or estimated duration of a life stage for a particular generation for all three beetle populations; some beginning and ending dates are averaged over the populations. White bars denote the duration of a life stage (or additional duration if after a black bar) for Crete beetles only. Gray bars denote the duration of a life stage averaged over the Tunisia and Uzbekistan beetles only.

**Phenology and Voltinism.** Observations of a caged, outdoor colony of *D. elongata* from Crete in 2003 showed that bud break of the saltcedar plants began 28 February 2003, and the first overwintered adult was observed feeding on the expanding leaves on 10 March 2003. Crete mating pairs were first seen on 17 March followed by the first egg mass on 4 April 2003, i.e., the beginning of the first summer generation. Based on additional observations of oviposition, larvae, and peaks in adult presence throughout the summer, we estimated that four to five generations of Crete beetles were possible in eastcentral Texas (starting approximately early April, late May, early July, early August, and early September). No egg masses were seen after mid-October, indicating that the adults had entered a reproductive diapause. More limited observations of the other *D. elongata* populations showed that Turpan adults had ceased reproduction and were buried in leaf litter in September, whereas some Tunisia and Uzbekistan adults were still reproducing into November.

Our more detailed observations and rearings in 2004 indicated that the phenology of the various populations of *D. elongata* was similar during the first half of the season at Temple, TX. Overwintered adults of all four populations began ovipositing the last week of March and continued to oviposit, under our experimental conditions, for 3–4 mo (Fig. 2). Approximately 50% of the overwintered adults had died by late May. Most adults were dead shortly after oviposition ceased, although some males and postreproductive females survived until early August. A combination of poor overwintering survival and few males (only four) of the Turpan beetle did not allow us to complete this aspect of the study for this population. However, we were able to confirm that the start of the first and second summer generations of Turpan beetles was similar to that of the other beetle populations.

Five summer generations were observed for the Crete, Tunisia, and Uzbekistan populations in 2004. An unsuccessful, partial sixth summer generation also occurred for *D. elongata* from Tunisia and Uzbekistan (Fig. 2). All life stages could be found throughout the growing season, with extensive overlap of up to three generations at any one time under our more limited rearing methods. In general, the beginning of each of the summer generations was as follows: first, late March; second, late May; third, late June; fourth, early August; fifth, early September; sixth, mid-October (Fig. 2). Adults (first cohort only) of the first three

Table 2.  Immature development and survival of five populations of *D. elongata* in laboratory tests, Temple, TX

| Life stage | Development (d) | | | | |
|---|---|---|---|---|---|
| | 2002 | | 2003 | | |
| | Crete, Greece | Fukang, China | Sfax, Tunisia | Karshi, Uzbekistan | Turpan, China |
| Eggs | 6.2 ± 0.1a (50) | 5.7 ± 0.1b (50) | 5.8 ± 0.1a (50) | 5.8 ± 0.1a (50) | 5.7 ± 0.1a (50) |
| First instar | — | — | 4.1 ± 0.1a (47) | 3.2 ± 0.1b (49) | 3.4 ± 0.1b (49) |
| Second instar | — | — | 3.3 ± 0.1ab (47) | 3.1 ± 0.1b (48) | 3.5 ± 0.1a (48) |
| Third instar | — | — | | | |
|   Active | — | — | 3.5 ± 0.1b (46) | 4.0 ± 0.1a (41) | 3.8 ± 0.1a (42) |
|   Prepupa | — | — | 2.8 ± 0.1a (43) | 3.1 ± 0.1a (36) | 2.9 ± 0.1a (41) |
| Pupa | — | — | 4.8 ± 0.0b (43) | 5.0 ± 0.1a (36) | 4.9 ± 0.0ab (39) |
| Total (neonate to adult) | 21.0 ± 0.2a (38) | 20.4 ± 0.2b (36) | 18.6 ± 0.2a (43) | 18.6 ± 0.2a (36) | 18.5 ± 0.2a (39) |
| | Percent survival (neonate to adult) | | | | |
| | 78.2 (64.8–89.2)a (12) | 74.5 (60.6–86.3)a (12) | 89.0 (77.8–96.6)a (5) | 73.0 (58.5–85.3)a (5) | 78.1 (64.3–89.3)a (5) |

Values are mean ± SE (*n*) except percent survival, which are back-transformed means from arcsine square root values, followed by 95% CIs in parentheses. 2002 and 2003 egg development based on 50 randomly selected observations (twice daily) of hatching from several egg masses. 2002 total development based on individuals used for mating pairs and percent survival based on 12 groups of 10 larvae each. 2003 larval/pupal development based on an initial 50 randomly selected first instars and percent survival based on five groups of 10 larvae each. Larvae provided saltcedar (*T. ramosissima*) under fluctuating temperatures (average, 28°C) and checked daily.

For each year, means within a row followed by the same letter are not significantly different (Fisher's protected least significant difference test, *P* > 0.05).

summer generations oviposited for ≈6–11 wk depending on the population (Fig. 2), and 50% of the adults had died after ≈6 wk. In all cases, a 4- to 7-d preoviposition period was observed.

The phenology of the *D. elongata* populations diverged beginning with the fourth summer generation. Crete beetle adults (first cohort only) of the fourth summer generation oviposited few egg masses over the course of 1 mo before ceasing oviposition in early October (Fig. 2). These beetles were presumably in reproductive diapause at this time; 90% of the adults were still alive in December. All the adults of the partial fifth summer generation of Crete *D. elongata* that emerged were in diapause (Fig. 2).

In contrast, Tunisia and Uzbekistan adults of the fourth summer generation continued to oviposit for up to 10 wk before all dying in November (Fig. 2). Adults of the fifth summer generation laid several egg masses over several weeks before entering diapause (Fig. 2); 83% of the adults of both populations were still alive in December. No larvae of the partial sixth summer generation survived, although one Tunisia beetle had developed to the prepupal stage. This indicates that larvae from any summer generation hatching from eggs laid from October on would not likely complete their development either.

**Larval Development and Adult Reproduction.** Some differences in development and reproduction were evident among *D. elongata* populations. For the 2002 laboratory test involving Crete and Fukang beetles, larval/pupal survival was not different, and only very minor differences in egg or total (neonate to adult) development times occurred (Table 2). Fukang females had a significantly shorter preoviposition period than Crete females. Fukang females also produced over twice as many egg masses that were one half the size of those produced by Crete females (Table 3). Otherwise, the Crete and Fukang populations did not differ in adult longevity or any other reproductive or population parameter (Tables 3 and 4).

The 2003 comparison of the Tunisia, Uzbekistan, and Turpan beetles showed no differences in days to egg hatch, larval/pupal survival, or total development times, although various minor differences in the duration of larval and pupal stages occurred (Table 2). Compared with the Tunisia and Uzbekistan populations, Turpan females had a shorter preoviposition period, laid twice as many egg masses, and laid smaller egg masses (Table 3). Also, the innate capacity for increase ($r_m$) was slightly greater for Turpan beetles than the Tunisia and Uzbekistan populations (Table 4). No differences in reproduction or population growth statistics occurred between the Tunisia and Uzbekistan beetles (Tables 3 and 4).

The four beetle populations, when allowed to oviposit in outdoor cages, laid egg masses that differed in size among all the beetle populations. Turpan and Crete females produced much smaller egg masses whereas Tunisia and Uzbekistan females laid larger egg masses (Table 5). However, substantial overlap in the number of eggs per mass occurred among beetle populations (Table 5). The percentage of eggs oviposited on saltcedar bark, as opposed to foliage, tended to increase from the Turpan and Tunisia beetles to the Uzbekistan beetle, but the differences were not significant in the 2003 test (Table 5). However, in the 2004 test, Uzbekistan females laid 35% of their eggs on the bark, which was much greater than the 0–1% of Crete and Tunisia beetles (Table 5). Eggs were found on both the main trunk and branches, including in bark crevices and the crotches of branches.

## Discussion

**Overwintering Survival.** Overwintering survival was consistent within beetle populations over two separate years for the Crete, Tunisia, and Uzbekistan beetles under eastcentral Texas conditions. Although we only tested the Turpan beetle for 1 yr, its early spring behavior and survival rate was similar to that of

**Table 3. Adult development and fecundity of *D. elongata* from five populations in laboratory tests, Temple, TX**

| Year of test *D. elongata* population | Duration (d) | | | |
|---|---|---|---|---|
| | Preoviposition period | Oviposition period | Total longevity, females[a] | Total longevity, males[a] |
| 2002 | | | | |
| Crete, Greece | 7.3 ± 0.4a (19) | 16.4 ± 2.3a (19) | 24.6 ± 3.1 (19) | 30.1 ± 3.1 (19) |
| Fukang, China | 4.3 ± 0.4b (18) | 17.6 ± 2.3a (18) | 23.2 ± 3.3 (17) | 33.8 ± 3.3 (17) |
| 2003 | | | | |
| Sfax, Tunisia | 4.5 ± 0.1a (19) | 13.0 ± 1.4a (19) | 19.1 ± 1.4 (19) | 17.3 ± 1.2 (25) |
| Karshi, Uzbekistan | 4.4 ± 0.1a (19) | 14.6 ± 1.4a (19) | 20.1 ± 1.4 (19) | 15.1 ± 1.2 (25) |
| Turpan, China | 3.6 ± 0.1b (18) | 13.1 ± 1.4a (18) | 17.9 ± 1.4 (18) | 18.1 ± 1.3 (23) |
| | Total eggs/female | Total egg masses/female | Eggs/mass | Percent hatch |
| 2002 | | | | |
| Crete, Greece | 280.7 ± 44.0a (19) | 15.4 ± 4.3a (19) | 17.6 ± 0.7a (19) | 77.9 (70.3–84.6)a (19) |
| Fukang, China | 283.1 ± 45.3a (18) | 35.9 ± 4.4b (18) | 9.0 ± 0.7b (18) | 76.7 (68.8–83.8)a (18) |
| 2003 | | | | |
| Sfax, Tunisia | 208.6 ± 24.1a (19) | 14.9 ± 2.4a (19) | 14.2 ± 0.4a (19) | 81.2 (75.5–86.3)a (19) |
| Karshi, Uzbekistan | 233.5 ± 24.1a (19) | 16.4 ± 2.4a (19) | 14.4 ± 0.4a (19) | 81.0 (78.7–89.0)a (19) |
| Turpan, China | 273.7 ± 24.8a (18) | 29.1 ± 2.4b (18) | 9.7 ± 0.4b (18) | 84.2 (75.3–86.1)a (18) |

Based on mating pairs of beetles fed saltcedar (*T. ramosissima*) as larvae and adults, reared under fluctuating temperatures (average, 28°C) and checked daily. Values are mean ± SE (*n*) except percent hatch, which are back-transformed means from arcsine square root values, followed by 95% CIs in parentheses.

For each year, means within a column (except longevity data) followed by the same letter are not significantly different (Fisher's protected least significant difference test, $P > 0.05$).

[a] For both years, neither beetle population nor population × sex was a significant effect ($P > 0.05$): 2002 sex—$F = 6.27$, df = 1,68, $P = 0.015$; 2003 sex—$F = 4.02$; df = 1,123; $P = 0.047$.

the Uzbekistan beetle. Adults of both the Turpan and Uzbekistan populations were active earliest of the four populations in the season (within the containers), at a time when saltcedar leaves were not yet available and subsequently suffered high mortality. Both of these populations originate from much higher latitudes (Uzbekistan, 38.86° N; Turpan, 42.86° N) than Temple (31.10° N). In contrast, Tunisia beetles (34.66° N), and especially Crete beetles (35.83° N), resumed activity in the containers at a later date, which was better synchronized with bud break. This was further confirmed for the Crete beetle by observations of the spring emergence of adults in a self-sustaining field cage colony. The Crete and Tunisia beetles currently seem to be better adapted than the Turpan and Uzbekistan populations to overwintering in more southern latitudes, i.e., south of 32° N latitude. We cannot say, however, if the Crete and Tunisia beetles will always have the best overwintering survival in other locations, because an alteration in the phenol-

ogy of both individual beetle populations and saltcedar growth with changes in latitude may allow for greater survival of the other populations tested.

**Phenology and Voltinism.** Despite apparent differences in initial spring activity among the four *D. elongata* populations that we observed in the overwintering study, all populations initiated oviposition at the same time, which seems to be closely tied to the availability of saltcedar foliage for adult feeding (Lewis et al. 2003b). The subsequent appearance of each new summer generation also was generally similar among the beetle populations, although we could track the Turpan beetle for only one complete generation. We therefore estimate that three summer generations, and potentially a partial fourth generation, may be possible for the Turpan beetle. This is based on our 2003 observation that adult beetles were in diapause by September, which may allow the earliest third-generation adults to begin reproducing (Fig. 2). Sha and Yibulayin (1993) had reported that

**Table 4. Population growth statistics (mean ± SE) for different populations of *D. elongata* in laboratory tests, Temple, TX**

| Year of test *D. elongata* population | Net reproductive rate ($R_o$) | Mean generation time (d, T) | Innate capacity for increase ($r_m$) | Population doubling time (d, DT) |
|---|---|---|---|---|
| 2002 | | | | |
| Crete, Greece | 96.3 ± 7.8a | 40.7 ± 1.7a | 0.113 ± 0.006a | 6.2 ± 0.3a |
| Fukang, China | 80.9 ± 7.8a | 38.3 ± 1.7a | 0.115 ± 0.006a | 6.1 ± 0.3a |
| 2003 | | | | |
| Sfax, Tunisia | 70.5 ± 9.7a | 33.8 ± 0.4a | 0.126 ± 0.002a | 5.5 ± 0.1ab |
| Karshi, Uzbekistan | 68.3 ± 9.7a | 34.6 ± 0.4a | 0.122 ± 0.002a | 5.7 ± 0.1a |
| Turpan, China | 89.4 ± 9.7a | 33.3 ± 0.4a | 0.134 ± 0.002b | 5.2 ± 0.1b |

Beetles reared on saltcedar (*T. ramosissima*) as larvae and adults under fluctuating temperatures (average, 28°C). Calculations based on values of 27.2 (Crete), 26.1 (Fukang), 24.4 (Tunisia, Uzbekistan), or 24.2 (Turpan) d for preadult development, *n* = 4 groups of females with 4–5 females per group.

For each year, means within a column followed by the same letter are not significantly different (Fisher's protected least significant difference test, $P > 0.05$).

Table 5.    Egg mass size and oviposition on saltcedar bark in outdoor cage tests of four populations of *D. elongata*, Temple, TX

| Beetle population | No. eggs/mass[a] | Percent eggs on bark, 2003[b] | Percent eggs on bark, 2004[c] |
|---|---|---|---|
| Turpan, China | 5.0 ± 0.5a (1–21) | 0.2 (−10.1 to 16.4)a | — |
| Crete, Greece | 8.8 ± 0.6b (1–22) | — | 0.0 (−1.9 to 1.9)a |
| Sfax, Tunisia | 18.2 ± 0.5c (1–36) | 2.4 (−2.1 to 19.5)a | 0.6 (−0.4 to 4.6)a |
| Karshi, Uzbekistan | 21.1 ± 0.5d (11–38) | 8.0 (0–30.8)a | 34.8 (22.4–48.4)b |

Oviposition on saltcedar (*T. ramosissima* x *T. chinensis*) by groups of 20 beetles (10 females and 10 males) held outdoors in small screen cages (68 by 53 by 85 cm) over 3 or 5 d. Values for eggs per mass are mean ± SE (range); values for percent eggs on bark are back-transformed means from arcsine square root values, followed by 95% CIs in parentheses.

Means within a column followed by the same letter are not significantly different (Fisher's protected least significant difference test, $P > 0.05$).

[a] Previously unpublished data from the paired-choice test (*Tamarix:Frankenia*) of Milbrath and DeLoach 2006a. $n = 6$ (Crete) or 10 (other populations).

[b] Previously unpublished data from the paired-choice test (potential host:nonhost) of Milbrath and DeLoach 2006a. $n = 2$ (Turpan) or 3 (other populations).

[c] Previously unpublished data from the no-choice test of Milbrath and DeLoach 2006b. $n = 5$ for all populations.

four generations were produced at Turpan, China, and Lewis et al. (2003b) had similarly predicted the potential for three or four generations in the southern United States. In contrast, the Fukang beetle produces two generations in the United States at field sites north of 38° N latitude (Lewis et al. 2003b, Bean et al. 2007).

The remaining *D. elongata* populations clearly are capable of reproducing continuously into September (Crete) or October (Tunisia, Uzbekistan) before the majority of adults enters diapause. Substantial overlap of different summer generations seems likely. In our study, usually two or even three generations were producing eggs simultaneously. The seminatural conditions of our study undoubtedly enhanced the survival of adult beetles and hence the duration of egg laying. Lewis et al. (2003b) reported that adults of the Fukang beetle typically live 4–6 wk in the field, which is one half the longevity that we observed for the other beetle populations. However, because we only followed the survival and reproduction of the first cohort of adults produced in each summer generation, we may be underestimating to some extent the duration of each summer generation. Regardless, the Crete, Tunisia, and Uzbekistan populations seem capable of providing up to 6–7 mo of defoliation of saltcedar by the larvae. We cannot definitively state that the longer duration of adult activity and oviposition by Crete beetles in each summer generation is an inherent trait of the population, because we were forced to rear the Crete beetles on different *Tamarix* species than the other populations. Nevertheless, the cessation of reproduction by Crete beetles currently is better synchronized than the other populations studied to the seasonal changes of central Texas.

**Larval Development and Adult Reproduction.** The only significant differences we observed in reproduction among beetle populations involved the number and size of egg masses, days to first oviposition, and site of oviposition. Although a direct comparison among all five populations studied is not possible, in no case did ovipositing more but smaller egg masses, i.e., Fukang and Turpan beetles, lead to differences in total fecundity with the other directly comparable *D. elongata* populations. Also, although both Chinese populations (Turpan and Fukang) began ovipositing sooner than

the others, this only led to a moderately higher innate capacity for increase ($r_m$) for the Turpan beetle. The relative delay in larval development and oviposition, and its subsequent effect on potential population growth, of the Fukang and Crete beetles in the 2002 test compared with the remaining populations in 2003 may have been the result of differences in larval rearing or plant quality. Nevertheless, the range of developmental and reproductive responses we observed for all populations are comparable to previous studies involving the Fukang and Crete beetles (Lewis et al. 2003b, Milbrath and DeLoach 2006a). It does not seem that any population is superior to any other in terms of the reproductive output of individual females. One practical issue that became apparent was that if the Uzbekistan beetle were to be released, any field monitoring for eggs must include examining the bark of saltcedar plants and not just the foliage, because Uzbekistan females were observed to lay up to one third of their eggs in masses located all over the trunk and branches. This pattern of oviposition was not observed in the other beetle populations.

Given the similar host specificity of the four populations (Milbrath and DeLoach 2006a, b) and the apparent lack of meaningful differences in reproduction reported here, the selection of which population(s) of *D. elongata* to release in the southern United States and northern Mexico may be determined, in part, by the results of our overwintering study, the number of generations produced in a growing season and therefore the period of defoliation of saltcedar expected, and the seasonal duration of oviposition relative to the onset of unfavorable fall conditions. The Turpan and Uzbekistan beetles seem to be poor choices, mainly because of the very high level of mortality experienced by overwintered adults. The high overwintering survival of the Tunisia beetle is partly offset by its relatively late diapause induction, which results in complete mortality of the last partial summer generation produced, at least in central Texas. However, it should not necessarily be disqualified for release. The Tunisia beetle may be better synchronized with saltcedar at sites further south, e.g., Zapata, TX (26.91° N), because we do not know what its phenology will be at such locations. Also, a change in the

Tunisia population's critical daylength for diapause induction may eventually occur after release, which would prevent late-season reproduction from occurring (Bean et al. 2007). At present, however, the Crete beetle seems best adapted to the conditions of central Texas, and possibly points further south, and has therefore been released at several sites (DeLoach et al. 2004).

## Acknowledgments

## References Cited

Baum, B. R. 1967. Introduced and naturalized tamarisks in the United States and Canada (Tamaricaceae). Baileya 15: 19–25.

Bean, D. W., T. L. Dudley, and J. C. Keller. 2007. Seasonal timing of diapause induction limits the effective range of *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae) as a biological control agent for tamarisk (*Tamarix* spp.). Environ. Entomol. 36: 15–25.

Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17: 15–26.

Cossé, A. A., R. J. Bartelt, B. W. Zilkowski, D. W. Bean, and R. J. Petroski. 2005. The aggregation pheromone of *Diorhabda elongata*, a biological control agent of saltcedar (*Tamarix* spp.): identification of two behaviorally active components. J. Chem. Ecol. 31: 657–670.

Crins, W. L. 1989. The Tamaricaceae in the southeastern United States. J. Arnold Arbor. Harv. Univ. 70: 403–425.

DeLoach, C. J., R. I. Carruthers, J. E. Lovich, T. L. Dudley, and S. D. Smith. 2000. Ecological interactions in the biological control of saltcedar (*Tamarix* spp.) in the United States: toward a new understanding, pp. 819–873. *In* N. R. Spencer (ed.), Proceedings of the X International Symposium on Biological Control of Weeds. Montana State University, Bozeman, MT.

DeLoach, C. J., P. A. Lewis, J. C. Herr, R. I. Carruthers, J. L. Tracy, and J. Johnson. 2003. Host specificity of the leaf beetle, Diorhabda elongata deserticola (Coleoptera: Chrysomelidae) from Asia, a biological control agent for saltcedars (*Tamarix:* Tamaricaceae) in the Western United States. Biol. Control 27: 117–147.

DeLoach, C. J., R. I. Carruthers, T. L. Dudley, D. Eberts, D. J. Kazmer, A. E. Knutson, D. W. Bean, J. Knight, P. A. Lewis, L. R. Milbrath, J. L. Tracy, N. Tomic-Carruthers, J. C. Herr, G. Abbott, S. Prestwich, G. Harruff, J. H. Everitt, D. C. Thompson, I. Mityaev, R. Jashenko, B. Li, R. Sobhian, A. Kirk, T. O. Robbins, and E. S. Delfosse. 2004. First results for control of saltcedar (*Tamarix* spp.) in the open field in the Western United States, pp. 505–513. *In* J. M. Cullen, D. T. Briese, D. J. Kriticos, W. M. Lonsdale, L. Morin, and J. K. Scott (eds.), Proceedings of the XI International Symposium on Biological Control of Weeds. CSIRO Entomology, Canberra, Australia.

DiTomaso, J. M. 1998. Impact, biology, and ecology of saltcedar (*Tamarix* spp.) in the Southwestern United States. Weed Technol. 12: 326–336.

Dudley, T. L., and D. J. Kazmer. 2005. Field assessment of the risk posed by *Diorhabda elongata*, a biocontrol agent for control of saltcedar (*Tamarix spp.*), to a nontarget plant, *Frankenia salina*. Biol. Control 35: 265–275.

Gaskin, J. F., and B. A. Schaal. 2002. Hybrid *Tamarix* widespread in U.S. invasion and undetected in native Asian range. Proc. Natl. Acad. Sci. U.S.A. 99: 11256–11259.

Gaskin, J. F., and B. A. Schaal. 2003. Molecular phylogenetic investigation of U.S. invasive *Tamarix*. Syst. Bot. 28: 86–95.

Herrera, A. M., D. D. Dahlsten, N. Tomic-Carruthers, and R. I. Carruthers. 2005. Estimating temperature-dependent developmental rates of *Diorhabda elongata* (Coleoptera: Chrysomelidae), a biological control agent of saltcedar (*Tamarix* spp.). Environ. Entomol. 34: 775–784.

Lewis, P. A., C. J. DeLoach, J. C. Herr, T. L. Dudley, and R. I. Carruthers. 2003a. Assessment of risk to native *Frankenia* shrubs from an Asian leaf beetle, *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae), introduced for biological control of saltcedars (*Tamarix* spp.) in the western United States. Biol. Control 27: 148–166.

Lewis, P. A., C. J. DeLoach, A. E. Knutson, J. L. Tracy, and T. O. Robbins. 2003b. Biology of *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae), an Asian leaf beetle for biological control of saltcedars (*Tamarix* spp.) in the United States. Biol. Control 27: 101–116.

Milbrath, L. R., and C. J. DeLoach. 2006a. Host specificity of different populations of the leaf beetle *Diorhabda elongata* (Coleoptera: Chrysomelidae), a biological control agent of saltcedar (*Tamarix* spp.). Biol. Control 36: 32–48.

Milbrath, L. R., and C. J. DeLoach. 2006b. Acceptability and Suitability of Athel, *Tamarix aphylla*, to the leaf beetle *Diorhabda elongata* (Coleoptera: Chrysomelidae), a biological control agent of saltcedar (*Tamarix* spp.). Environ. Entomol. 35: 1379–1389.

Robinson, T. W. 1965. Introduction, spread and areal extent of saltcedar (*Tamarix*) in the western states. U.S. Geological Survey, Washington, DC.

SAS Institute. 2004. SAS/STAT user's guide, version 9.1. SAS Institute, Cary, NC.

Sha, P., and W. Yibulayin. 1993. Preliminary study on *Diorhabda elongata deserticola* Chen: biological characteristics and control methods. Xinjiang Forest. Sci. Technol. J. 1: 7–10.

Zavaleta, E. 2000. The economic value of controlling an invasive shrub. Ambio 29: 462–467.